PHENOTYPING IN RYE LANDRACE AND REGENERATION FREQUENCY STUDY IN WHEAT × RYE F_1 HYBRID SEEDS.

ABSTRACT

The present research aimed to study the phenotyping characterization of a high-altitude Himalayan rye landrace and its crossability with wheat genotypes and investigate the regeneration frequency of wheat × rye F1 hybrid seeds. The study was conducted during the rabiseason of 2022-2023 at the Agriculture Research Farm, Department of Plant Breeding and Genetics, Lovely Professional University, Phagwara, Punjab. The phenotypic characterization of the Himalayan rye landrace revealedseveral adaptive traits that were found suitable for cultivation in the northwest Indo-Gangetic plains. Furthermore, the study investigated the crossability of the Himalayan rye landrace with various wheat genotypes, including 4 genotypes of bread wheat HD2967, UNNAT 343, WH1105, and PBW502 and 4 genotypes of durum wheat PDW291, PDW274, PBW34, and WHD943. Among the germination percentage of all 8 genotypes, 4 in Bread wheat and 4 in Durum wheat was observed. Due to its self-incompatibility, Rye cannot be used to develop inbred lines directly. However, the doubled haploid approach can produce inbred lines in the rye, facilitating the extension of genetic bases and introducing alien chromatin to identify stress tolerances.

Keywords –Wide hybridization, wheat×Rye, Embryorescue,Crossbility percentage, Regeneration Frequency

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is an important cereal crop of the world contributing around 20% of the calories of the human diet with its production reaching ~770 Mt in 2020-2021 (Hawkesford, 2013, FAO 2021). Durum wheat (*Triticum turgidum* ssp. Durum). (2n=4x=28, AABB) is a significant crop globally, ranked tenth in importance. It is mainly cultivated in West Asia, North and East Africa, the Great Plains of North America, India, and Eastern and Mediterranean Europe, covering approximately 10% of the total wheat area. Typically, durum wheat production constitutes 5% of the total wheat production, with a worldwide cultivation area of 16 million hectares (Beres *et.al*., 2020). Rye is a cereal crop

primarily grown in temperate areas (Sun *et.al*., 2022). Its genome 2n=14 places it in the Poaceae family. Rye's genetic material is 8100 Mb in size. (Li *et.al*., 2011). History believes that rye originated in southwest Asia (Gyulai *et.al.* 2014). Recent studies revealed that rye underwent a particular evolution, changing from a wild plant to a weed and then from a weed plant to an agricultural plant (Gyulai *et.al*., 2014). Rye is one of the most utilized relatives of wheat in this process (Kim *et.al*., 2004). In 1873 Alexander Wilson first managed to manually fertilize the female stigma of wheat flowers with rye pollen (male gametes) but found that the resulting plants were sterile. One of the first published reports on the use of triticale as a grain feed is (Meister, 1921). The first variety of wheat x rye F_1 was developed in laboratories during the late 19th century in Scotland and Germany by Meister in 1921. It was developed for the yield potential and grain quality of wheat with disease and environmental tolerance including soil conditions of rye. Wheat has been enhanced by introducing rye's genes into wheat with the help of substitution lines and translocation lines. When introduced into wheat, rye has various resistant genes that give wheat protection against several diseases. The hybrid plants were developed by crossing wheat x of rye and it has a lot of importance as they withstand the cold winter season better than Bread wheat (Meister, 1921). The major importance of triticale lies in its protein. Reported protein values range from 12 to 22%. triticale hybrids were all amphidiploid (Levitzky and Benetzkaja, 1930) which means having a complete diploid chromosome set from each parent. It is an allotetraploid possessing four times the chromosomes in haploid organisms (Neubauer, 1966).

Objectives:

1. Phenotypic characterization of a high-altitude Himalayan rye landrace in northwest Indo-Gangetic plains.
2. Crossability percentage in the (Bread 6x) and (Durum 4x) wheat.
3. Comparison of the regeneration frequency in different wheat × rye F_1 hybrid combinations.

MATERIAL AND METHODS

The present research entitled Phenotyping of rye landrace and Regeneration frequency study in wheat × rye F_1 hybrid seedswas conducted in the experimental fields of the Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara, Kapurthala, Punjab, India at coordinates latitude 31019’32’’N, a longitude 75034’45’’E, and an altitude of 243 meters above sea level. The research is done in

the Rabiseason of 2022-2023. Punjab is in the western part of India which is the subtropical region. The climate in this region is humid subtropical, with cool winters and long hot summers. The summer season lasts from April to June, and the winter season lasts from November to February. Summer temperatures range from 25°C to 48°C on average. Winter temperature ranges from 1 °C to 18 °C. The soil is slightly acidic, with a pH range of 5.6-6.4, and the soil texture is sandy loam. The regeneration frequency was done under controlled conditions at the Plant Breeding Laboratory, Department of Genetics. The Experimental materials that were used were Four Bread wheat genotypes namely WH1105, PBW502, HD2967, and Unnat343. Four Durum wheat genotypes namely PDW291, PDW274, PBW34, and WHD943 Rye Genotype Himalayan rye landrace, M.S media are collected from the Department of Agriculture, Punjab. The sowing of genotypes was done at three different intervals i.e. (5^{th} November 2022). Each genotype was sown in 2 lines with a total of 8 lines, and 4 genotypes of Durum wheat (4x) in 2 lines with a total of 8 lines, with a length of 2 meters. 2^{nd} and the 3^{rd} block sowing were done exactly in the same pattern as in the first block. On 28^{th} November 2022 and 14^{th} December 2022. Rye genotypes (Himalayan rye) are sown in 3 × 3meter lengths in two blocks of 10 lines. The removal of immature anthers without causing stigma damage is known as emasculation. Wheat plants are emasculated in the evening two to three days before anthesis it was done in the evening time. The rye pollen is available from 9.00 a.m. to 11.00 a.m. For calculating the crossability percentage of crosses the formula was used.

$$Seed\ set\ \% = \frac{no.\ of\ seeds\ formed}{no.of\ florets\ pollineted} \times 100$$

$$Crossabilty\ \% = \frac{no.\ of\ plants\ obtained}{no.of\ florets\ pollineted} \times 100$$

$$Regenration\ frequency\ \% = \frac{no.\ of seeds\ germinated}{no.of\ seeds\ sown} \times 100$$

The composition was used for preparing MS. Media for the embryo rescue process.

MS. media + IAA(1mg/l) + kinetin(0.5mg/l). MS. media + 2,4- D(1mg/l). Agar = 7gm per 100ml. 70gm per 1000ml. total of 63 gm agar is added in MS. media. After the preparation of MS media, the crossed spike of durum wheat is collected 15 to 20 days after the crossing and

the pseudo seeds formed were collected with the help of forceps and transferred into the Petri plate filled with distilled water and embryo rescue is done.

RESULTS AND DISCUSSION.

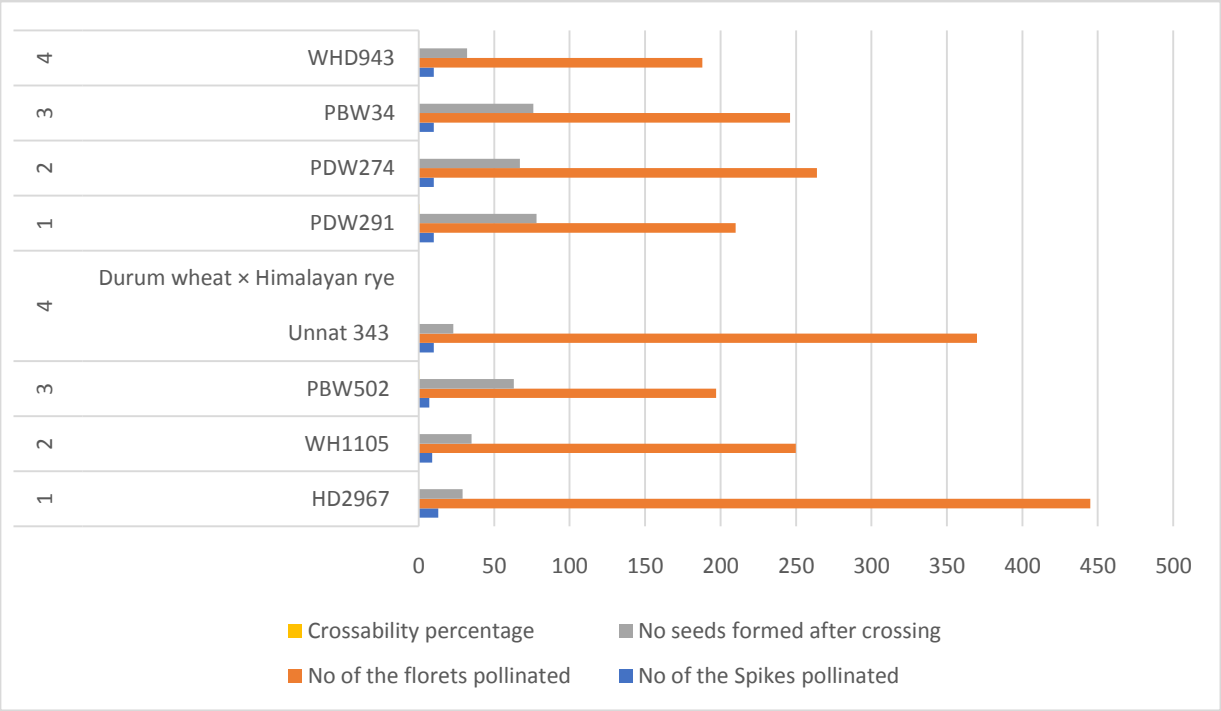
Crossability percentage of Bread and Durum Wheat × rye

The crossability percentage of 4 Bread kinds of wheat with that of Himalayan rye was calculated and the results are presented. A total of 1262 florets were pollinated as per the procedure described in the material and methods from which 150 seeds were obtained. The average percentage crossability of all Bread genotypes was 14.67%. (Gitta Oettler, 1983) found similar results when he crossed bread wheat × Rye. The crossability percentage lies between 20.7% to 37.5%. The crossability percentage ranged from 6.21% to 31.97% among the 4 genotypes *viz.* HD2967, WH1105, PBW502, Unnat 343. Genotype PBW502 gave the maximum percentage viz., 197 florets 63 seeds were formed followed by WH1105 viz., 250 florets 35 seeds were formed, and come next genotype HD2967 of 445 florets 29 seeds are formed and finally Unnat 343 a total of 370 florets 23 seeds are formed. It was observed that genotype PBW502 gives maximum seeds when crossed with Himalayan rye and genotype unnat343 has less seed formation compared to all other genotypes. Similarly, Oettler, G. 1983 found the formation of seed when crossed Bread wheat x rye.

The crossability percentage of 4 Durum kinds of wheat with that of Himalayan rye was calculated and the results are presented. Unlike Bread wheat no seed set was observed in Durum kinds of wheat and the hybrid plants must be obtained with the help of embryo rescue techniques. Bajaj et.al., 1978 found similar results that the optimal outcome to produce Bread triticale from an otherwise incompatible cross between *Triticum durum* × *Secale cereale* would be predicted from hybrid embryos that were removed 16 to 18 days after pollination and cultivated carefully on a layer of macerated, immature endosperm dispersed over an agar nutritional medium with IAA, kinetin, and casein hydrolysate. A total of 908 florets were pollinated as per the procedure described in the material and methods from which 281 embryos were obtained. The average percentage of embryo formation frequency was 30.93%. The embryo formation frequency percentage ranged from 17.02% to 50.47% among the 4 genotypes *viz.* PDW291, PDW34, PDW 274, and WHD943. Genotype PDW291 gave the maximum percentage viz., 210 florets pollinated and the 106 embryos obtained with a percentage of 50.47% followed by PDW34 viz., 246 florets 76 embryos were formed, with an

embryo formation percentage of 30.89%, and then genotype PDW 274 viz., 264 florets 67 embryos were formed with embryo formation percentage 25.37% and the last come to the genotype WHD943 with total 188 florets the total embryos formed is 32 with embryo formation percentage of 17.02%. It was observed that genotype PBW291 has produced more embryos and the genotype WHD943 has produced fewer embryos.

Chart. 1Crossbility percentage of bread wheat and durum wheat × rye



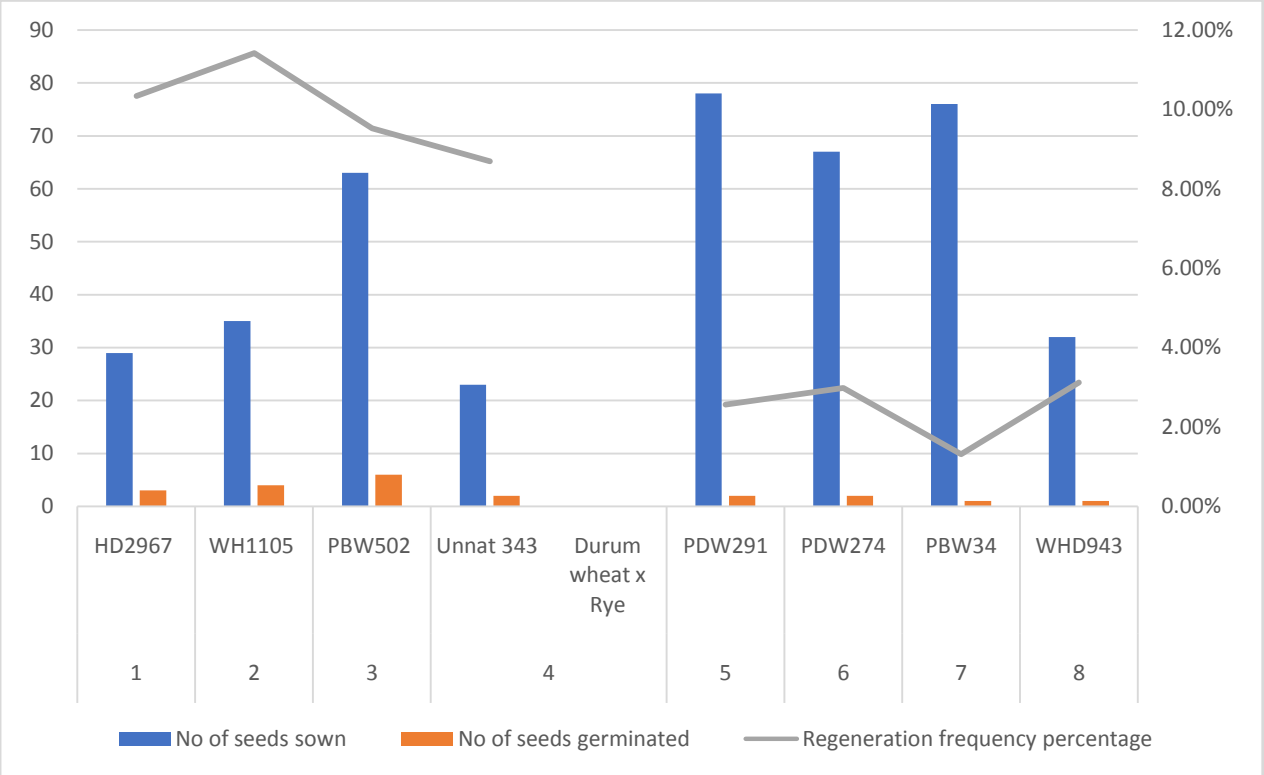
Regeneration frequency of Bread and durum wheat × rye seeds

The regeneration frequency percentage of Bread crossed with rye seeds was sown. the data calculated and presented. To check the regeneration frequency percentage of the Bread wheat crossed seeds as the crossed seeds are sown in the pots by providing all the requirements for germination and checking whether germination is there in the seeds are not. And the percentage is calculated by the formula mentioned above in the materials and materials. The total no of seeds sown was 150 and the seeds germinated was 15. The regeneration frequency lies between 11.42% to 8.69% among the 4 genotypes. All bread wheat genotypes have an average regeneration frequency of 9.99%. The seeds of genotype PBW502 have more germination with 6 seeds followed by WH1105 with 4 seeds and HD2967 with 3 seeds germination and last with Unnat343 with 2 seeds. The regeneration frequency is different when compared to the seed's germination as the percentage was higher for the genotype WH1105 at 11.42% and with the second place HD2967 with a percent of

10.34% and next PBW502 with a percentage of 9.52% and the was Unnat343 with percentage of 8.69%.

The regeneration frequency percentage of durum wheat crossed with rye seeds was sown. the data calculated and presented. To check the regeneration frequency percentage of the embryos obtained by crossing durum wheat with rye we need to do embryo rescue by preparing an MS media by following the procedure described in the material and methods and providing all the requirements for germination and checking whether the embryo has formed into plantlets are not. And the percentage is calculated by the formula mentioned above in the materials and materials. The results obtained after embryo rescue were discussed and the plantlets formed was 2 in the genotype PDW291 followed by PDW274 with 2 plantlets PBW34 with 1 plantlet and the genotype WHD943 with 1 plantlet. All the durum wheat genotypes have an average regeneration frequency of 2.32%. The plantlets regeneration frequency percentage has maximum in WHD943 with 3.12% followed by PDW274 with 2.98% and next PDW291 with 1.88% and last PBW34 with 1.31%.

Chart. 2 Regeneration Frequency of Bread and Durum Wheat x Rye



SUMMARY

In bread wheat, the genotype PBW502 produced more seeds with 106 out of 197 florets pollinated highest number of seeds are formed in this genotype as compared to other genotypes and the crossability percentage is higher for this genotype at 31.97%. the regeneration frequency of this genotype is 9.52 %. In bread wheat, the genotype WH1105 produced 35 seeds out of 250 florets pollinated the crossability percentage of this genotype is 14% and the regeneration frequency percentage is 11.42% as compared to another genotype the regeneration frequency is higher for WH1105. In durum wheat, the genotype PDW291 embryo obtained was 106 out of 210 florets pollinated and with a crossability percentage of 50.47%. The regeneration frequency of this genotype is 1.88%. In durum wheat, the genotype WHD943 embryo obtained was 32 out of 188 florets pollinated and the embryo formation percentage is 17.02%. The regeneration frequency is 3.12%.

Conclusion.

The phenotypic characterization of a high-altitude Himalayan rye landrace in the northwest Indo-Gangetic plains revealed its adaptive traits, including compact growth habits, resilient leaf structure and strong stemshave a high crossability with *Triticum aestivum* and *Triticum durum* without the application of any Phyto-hormones-furthermore,the crossability of Rye is extremely self-incompatible, hence the only way to create inbred lines was using doubled haploid technology. The genotypes PBW502, WH1105, PDW291, and WHD943 have given better results after crossing with the Himalayan rye landrace.

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